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Strain-Dependent Susceptibility to MPTP and MPP^+-Induced Parkinsonism Is Determined by Glia

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KEY WORDS Parkinson's disease; reactive oxygen species; dopamine; cell culture

ABSTRACT Parkinson's disease (PD) is a debilitating neurological disorder that strikes approximately 2% of people over age 50. Current hypotheses propose that the cause of PD is multifactorial, involving environmental agents and genetic predisposition. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces parkinsonism in many species, including humans and shows strain specificity in mice. The mechanism of strain specificity, however, remains unknown. Using novel chimeric murine substantia nigra cultures we demonstrate that sensitivity to MPTP is conferred by glia and that it does not involve the MAO-B conversion of MPTP to MPP^+. C57Bl/6J dopaminergic neurons exposed to MPP^+ demonstrated a 39% loss when cultured on C57Bl/6J glia compared with 17% neuron loss when cultured on resistant SWR/J glia. Similarly, SWR/J neurons exposed to MPP^+ demonstrated a 4% loss when cultured on SWR/J glia, but a 14% loss when cultured on sensitive C57Bl/6J glia. The identification of glia as the critical cell type in the genesis of experimental Parkinsonism provides a target for the development of new anti-parkinsonian therapies. *GLIA 34:73–80, 2001.*

INTRODUCTION

Idiopathic Parkinson's disease (PD) is characterized by loss of pigmented dopaminergic neurons located in the midbrain substantia nigra pars compacta (SNpc), followed by generalized gliosis (Jellinger, 1996; Renkawek et al., 1999). PD patients also exhibit signs of oxidative stress caused by the metabolism of dopamine. Oxidation of dopamine leads to formation of bioreactive dopamine intermediates and reactive oxygen species (ROS), increased lipid peroxidation, decreased glutathione and glutathione peroxidase, increased iron, decreased ferritin, and altered mitochondrial function (Jenner, 1998).

Although the pathology of PD is well understood, its etiology is unknown. Current hypotheses posit that PD pathology is initiated by the interaction of an environmental agent with a genetic sensitivity to this compound; and it is only when these two susceptibility factors intersect that the disorder is manifested (Checkoway and Nelson, 1999; Kondo et al., 1973; Stoessl, 1999). One xenobiotic agent that induces parkinsonism in a number of vertebrates, including mice, is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Mari and Bodis-Wollner, 1997). MPTP enters the brain glial cells through a number of mechanisms, including monoamine (Brooks et al., 1989) and glutamate (Hazell et al., 1997) transporters or pH-dependent antiporters (Kopin, 1992). Once in these cells, MPTP is converted to $MPDP^+$ and MPP^+ via intracellular monoamine oxidases. MPP^+ is then released from glia and taken up by neuronal dopamine transport-

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ers where it interferes with Complex I respiration in the electron-transport chain (Jenner and Marsden, 1993).

Different mouse strains show varying responses to MPTP, falling into either a "sensitive" (indicating greater than 50% SNpc neuron loss) or "resistant" (showing a less than 25% SNpc neuron loss) category (German et al., 1996; Hamre et al., 1999). Crosses of the MPTP-sensitive C57Bl/6J strain with the MPTP-resistant SWR/J strain of mice have indicated that the susceptibility trait is dominant and that one major locus is probably responsible for the susceptibility trait (Hamre et al., 1999). However, it is unknown whether the critical cells in the progression of MPTP or MPP^+-induced PD are neurons or glia. The present study uses a novel chimeric neuron/glial in vitro system to demonstrate that MPTP and MPP^+-induced dopaminergic cell loss is modulated by glial cells. The identification of a major glial effect may have important implications in the understanding and future treatments of PD.

MATERIALS AND METHODS

Generation of Midbrain, Single Genotype Substantia Nigra, and Chimeric Substantia Nigra Cultures

All the animal studies reported in this article were approved by the St. Jude Children's Research Hospital Animal Care and Use Committee. The methods used to generate midbrain and substantia nigra (SN) cultures were modified from Cardozo (1993). Brains from postnatal days 0–5 (P0–5) C57BL/6J or SWR/J mice were removed and placed in dissociation media (DM). Whole midbrains (Fig. 1A) or SN (Fig. 1B) were dissected, placed in fresh DM, minced into small pieces, and then incubated at 37°C (2 × 30 min) in papain (Worthington Biochemical, Freehold, NJ) . The tissue was rinsed and triturated, and the cell suspension was layered over plating media (PM) with 10 mg/ml bovine serum albumin (BSA) and 10 mg/ml ovomucoid albumin. The cell suspension was spun and then resuspended in PM with 2% rat serum, counted using Trypan Blue, and plated at 200,000 cells/cm^2 in Lab-Tek (TM) 4-well Permanox chamber slides (2 wells of C57Bl/6J cells and 2 wells of SWR/J cells/slide) coated with laminin (200 mg/ml, Collaborative Biomedical Products) and poly-D-lysine (200 mg/ml, Collaborative Biomedical Products) 1:1 (vol: vol). Cells were maintained in an incubator at 37°C, 5% CO_2, and fed 2–3 times per week with feeding media.

For chimeric cultures, SN cells grown on preplated SN glia were produced using a variation of the above-stated procedures. SN glial feeder layers from P0–5 C57BL/6J or SWR/J mice were produced as above, but cells were instead plated at 20,000–50,000 cells/cm^2 and fed with PM containing 2% rat serum (RS) and 8% fetal bovin serum (FBS) to promote glial proliferation and neuronal death. Imm diately before the addition of the neurons, the glial feeder lay rs were rinsed with PM without serum, followed by the addition of 0.5 ml of plating media with 2% RS. SN cells from C57BL/6J or

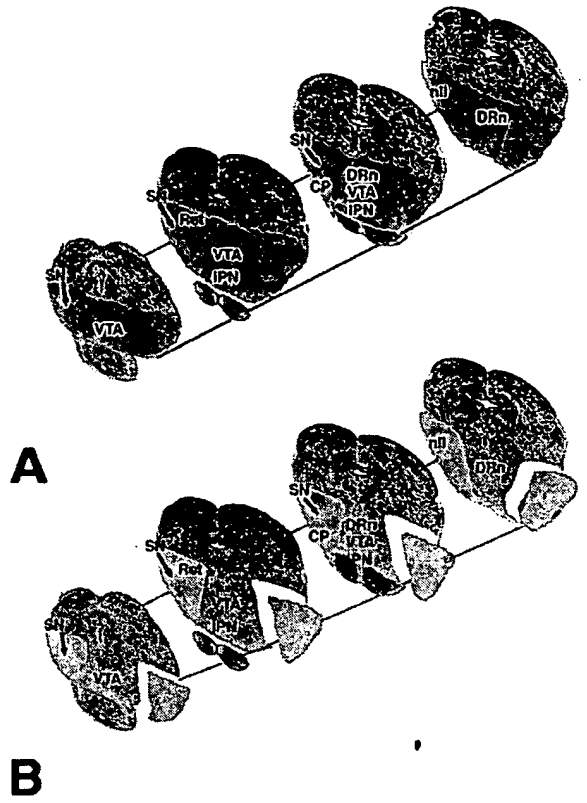

Fig. 1. A: The region in light gray is the area typically dissected when preparing midbrain cultures. This region contains all of the dopaminergic cells of the substantia nigra (SN) as well as dopaminergic cells and noradrenergic cells of the ventral tegmental area (VTA), reticular nuclei (ret), interpeduncular nucleus (IPN), dorsal red nucleus (DRn), nucleus of the lateral lemniscus (nll) and cells of the cerebral peduncle (cp). B: In our modified dissection, only the cells located in the ventrolateral parts of the midbrain are dissected. Cells in this region are enriched for substantia nigra (both pars compacta and pars reticulata). The dissection also has cells from the reticular nuclei and cells from the nucleus of the lateral lemnsicus. A large proportion of the cells dissected in A are not used, including those from the VTA, IPN, and the DRn. C: Typical appearance of TH^+ cells grown in the chimeric cultures. Dopaminergic cells (arrows) are usually multipolar, with a large cell body and large pale nucleus. These cells appear to grow preferentially upon the glial mat (arrowheads).

SWR/J mice were isolated and plated at 250,000 cells/well onto the glial feeding layers. At 24 h postplating, the cultures were fed with feeding media with 2% RS and Ara-C (2 μM) to prevent glial proliferation of the freshly plated cells. Thereafter, Ara-C (10 μM, final concentration) was added at each feeding. C57Bl/6J and SWR/J cell cultures used for comparisons in this study were generated in parallel in all culture paradigms.

MPTP and MPP+ Administration

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, Sigma Chemical Company, St. Louis, MO) or 1-methyl-4-phenylpyridinium (MPP^+, Sigma) were added to cultures 7–10 days after neurons were plated onto glia. MPTP or MPP^+ were dissolved in feeding media with 2% rat serum and added directly to the cultures. The final concentration of the MPTP or MPP^+ in media was 50 nM. Two feedings of MPTP or MPP^+, spaced 2 days apart, were necessary to achieve the desired toxic effect.

Seven days after MPTP or MPP^+ administration, cultures were rinsed 3× with Tris-buffered saline (TBS), fixed for 10–15 min in 4% buffered paraformaldehyde, and rinsed 3× with TBS. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide in methanol in 1× TBS for 2 × 15 minutes. Cells were permeabolized with 1% Triton X-100, 5% BSA in TBS for 2 × 15 min. Dopaminergic neurons were visualized using a polyclonal antibody directed against tyrosine hydroxylase (Eugene Tech International (Ramsey, NJ, or Pel-Freeze, Burlingame, CA), each 1:500. Cultures were incubated with primary antibody overnight at 4°C. On day 2, cultures were rinsed 3× with TBS, followed by application of secondary antibody (goat-α-rabbit) and amplification with avidin-biotin (ABC Elite Peroxidase kit, Vector Laboratories, Burlingame, CA). Final visualization of the immunoreactive neurons was made using diaminobenzidine (DAB, Vector) as a chromagen.

All TH^+ cells having the previously described characteristics of SNpc neurons (Hamre et al., 1999) (Fig. 1C) from each culture were counted on a Zeiss Axiolab microscope at a magnification of 200×.

One of the hallmarks of cell culture preparation and maintenance is the variability that occurs between individual cultures. To control for variability between experimental cultures, all MPTP treatments were performed in culture wells that were present on the same culture slide as control cultures wells. Once cells were counted in each well, the number of TH^+ cells in the MPTP-treated cultures from each genotype was compared with th adjacent nontreat d culture of th sam genotype. Sinc an identical number of cells generated from the same brains wer plated on a singl slid , these cultures were directly compared to determine the percentage cell loss after MPTP. The percentage cell loss after MPTP was then summed from all C57Bl/6J, Swiss–Webster or mixed cultures and a mean percentage cell loss was calculated. We also used statistical analyses (see below) that took into account all of the variability in raw cell numbers that occurred between individual cultures. As an additional control, culture pairs were only used in the analysis if they contained a minimum of 250 TH^+ neurons per control culture well.

TABLE 1. Percentage neuronal loss in SNpc cultures

Group	Culture genotype	n	% cell loss	Statistics
Midbrain cultures				
1	C57Bl/6J	20	3 ± 1	1 vs 2
2	SWR/J	11	2 ± 1	
SNpc cultures				
3	C57Bl/6J	14	50 ± 5	3 vs 4*
4	SWR/J	7	10 ± 3	
Glial genotype	**Neuronal genotype**	**n**	**% cell loss**	**Statistics**
Chimeric cultures treated with MPTP				
5 C57Bl/6J	C57Bl/6J	14	30	5 vs 6**
				5 vs 7*
				5 vs 8†
6 SWR/J	C57Bl/6J	15	11	6 vs 7‡
				6 vs 8 n.s.
7 SWR/J	SWR/J	11	4	7 vs 8 n.s.
8 C57Bl/6J	SWR/J	15	14	
Chimeric cultures treated with MPP^+				
9 C57Bl/6J	C57Bl/6J	7	39	9 vs 10‡
				9 vs 11‡
				9 vs 12‡
10 SWR/J	C57Bl/6J	9	17	10 vs 11 n.s.
				10 vs 12 n.s.
11 SWR/J	SWR/J	5	4	11 vs 12 n.s.
12 C57Bl/6J	SWR/J	8	14	

*$P < 0.0001$.
**$P < 0.05$.
†$P < 0.001$.
‡$P < 0.01$.
n.s., not significantly different.

Statistics

To estimate the toxicity of MPTP, the data were expressed by the following ratio of cell loss:

$$r_i = (x_i - y_i)/x_i$$

where x_i was the number of TH^+ cells in control cultures, y_i was the number of TH^+ cells in MPTP-treated cultures. We then examined the data, using several statistical techniques. Plots provided important information on outliers and distributions; numerical summaries gave a synopsis of the data. Sample means and standard error of the mean (SEM) calculated for the 12 groups are displayed in Table 1. The normality of the data was determined by the Wilk–Shapiro test. We applied the *F*-test for population variance equality. Based on exploratory data analysis, the following methods were mployed to test th hypothesis that the MPTP effect was determined by glial genotyp . Comparisons between th groups wer mad with Welch modified two-sample *t*-test (Welch modification is used when population variances are not equal), or nonpara-

metric Wilcoxon rank-sum test if the results were not normally distributed. We compared groups 1 and 2, groups 3 and 4, and all possible combinations of two sets of observations for groups 5–8, and for groups 9–12. The null hypothesis for the Welch modified test is that the population means for groups 1 and 2 are the same. The Wilcoxon rank-sum test was used to test whether two sets of results come from the same distribution. The hypothesis testing was done as two-tailed. Statistical significance was assumed if $P < 0.05$. In addition, the general linear model approach (GLM) to analysis of variance (ANOVA) (Milliken, 1992; Searle, 1987) was applied to check whether the glia or neurons, or both, had a significant effect on the cell loss after MPTP administration in groups 5–8. Both factors—glia and neuron—had two levels: C57Bl/6J and SWR/J mouse strains. To allow for interactions, we added an additional interaction term *glia*neuron* into the model with two main effects: *glia* and *neuron*.

RESULTS

In order to determine whether the glial cell, the neuron, or an interaction of the two was responsible for the observed dopaminergic cell death, we developed an in vitro method that recapitulated the previously observed in vivo strain differences (Hamre et al., 1999). Whole midbrain cultures, which include cells of the substantia nigra pars compacta (SNpc) , substantia nigra pars reticularis (SNpr), ventral tegmental area (VTA), retrorubral nucleus (RRn), nucleus paranigralis (nPN), and red nucleus (RN) (Fig. 1A) yielded no significant TH^+ cell death after MPTP administration in either the MPTP-sensitive C57Bl/6J or resistant SWR/J strains (Table 1). This is in contrast to in vivo experiments that demonstrated a loss of 63% and 10% of SNpc neurons after MPTP administration in C57Bl/6J and SWR/J mice, respectively (Hamre et al., 1999). The lack of in vitro cell death was likely due to the numerous MPTP-resistant dopaminergic and noradrenergic neurons that were present in the dissected tissue (Hung and Lee, 1996; Schneider et al., 1987).

In order to enrich for SN neurons, we generated cultures from postnatal animals in which the ventral midbrain populations had fully migrated to their final positions (Bayer et al., 1995) (Fig. 1B). On the basis of cell counts of these areas (Baker et al., 1980; Lieb et al., 1996), we estimated that our cultures were enriched 3–6-fold for SN neurons compared with whole midbrain preparations. When we examined cell loss in the SN cultures generated in parallel from both strains of mice, we observed that C57Bl/6J mice had a loss of 50 ± 5% of TH^+ neurons, while cultures derived from SWR/J mic showed a loss of 10 ± 3% (Table 1). Th percentage cell loss seen in our in vitro system was approximately 80% of the in viv value. Th 20% difference was likely attributable to the presence of residual MPTP-resistant cell populations that could not be separated during the ventrolateral dissection. In addition, our strain-specific cell loss was observed using a concentration of MPTP that was 200-fold less (50×10^{-9} vs. 10×10^{-6}) than other studies that examined the effects of MPTP in vitro (McNaught and Jenner, 1999; Sanchez-Ramos et al., 1988). It is likely that the toxicity seen using lower concentrations of MPTP than previous studies was due to the enrichment of substantia nigra neurons versus other catacholaminergic neurons present in whole midbrain preparations (Johannessen et al., 1989), which have been shown to be less sensitive to MPTP.

Our culture system was further modified to produce chimeric cultures in which SN neurons of the sensitive C57Bl/6J strain were grown on glia derived from the SN of resistant SWR/J strain and vice-versa. These chimeric cultures were generated by growing pure glial mats from C57Bl/6J or SWR/J mice, using a high serum protocol where glia proliferate in the absence of neurons. Once glia reached confluence, SN cells from the same or different genotype were plated onto the glial monolayer. After 7–10 days, the chimeric cultures were treated with MPTP, and 7 days later, TH^+ cell survival was determined.

Culturing neurons on established glia produced SNpc neurons (Fig. 1C) that quickly extended processes with an extensive arbor. Although significant differences in the strains' responses to MPTP remained, the percentage of cell death was less than that of SNpc cultures produced in a single step (Table 1). In the chimeric cultures, in which the effects of individual cell types can be differentiated, we found that the glia exerted a direct and controlling influence on cell death. When C57Bl/6J cells were plated onto confluent SWR/J glia, the amount of cell death was significantly reduced from the C57Bl/6J cells grown on C57Bl/6J confluent glia. Similarly, when SWR/J cells were plated on confluent C57Bl/6J glia, the amount of cell death was increased from the SWR/J cells plated on SWR/J confluent glia (Table 1, Fig. 2A).

To determine whether there were neuronal–glial interactions, we used a general linear model approach t ANOVA. We found that the glial effect on cell death was independent of the neuronal effect ($P = 0.51$). Th use of SWR/J glia resulted in a lower ratio of TH^+ c ll loss than C57Bl/6J glia for neurons of both genotypes, making the effect of glia significant at $P \leq 0.0001$. There is also a neuronal effect ($P \leq 0.03$), since SWR/J neurons have a lower ratio of cell loss than that of C57Bl/6J neurons on glial monolayers from either genotype. Statistical analyses allow us to make an actual comparison between the glial and neuronal effects. Using a measure of the difference between estimated effects of C57Bl/6J glia minus SWR/J glia over the difference between estimated effects of C57Bl/6J neurons minus SWR/J neurons, we calculated an R value of 2.95. This is interpreted to show that the effects of glia are approximately three times that of neurons; or glial ffects wer responsible for approximately 75% of the neuronal cell loss compared with 25% cell loss du to neuronal effects.

Monoamine xidase-B within glia depr tonates MPTP t its toxic metabolit , MPP^+ (Di Monte et al.,

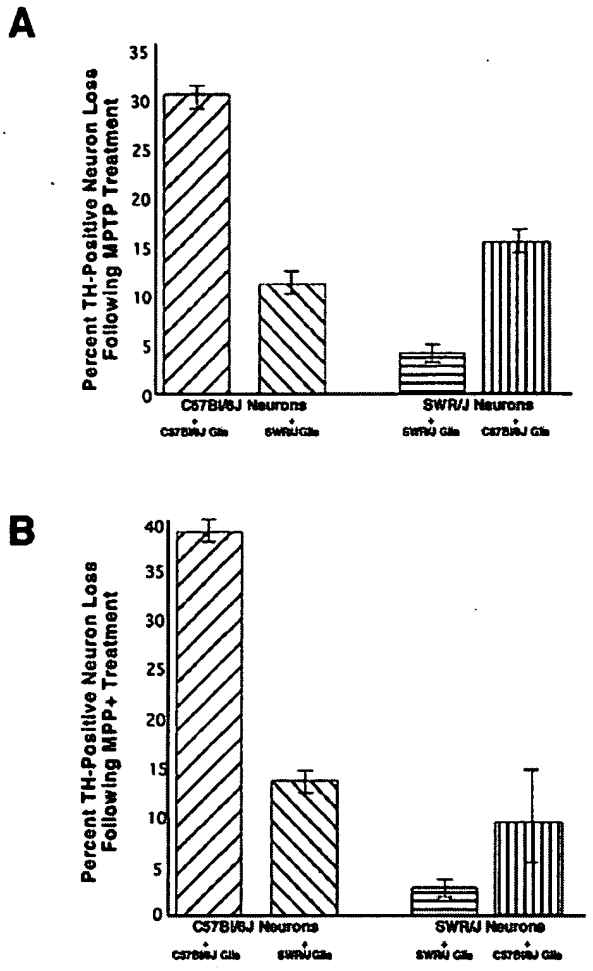

Fig. 2. Changes in cell death are dependent on the glial genotype. A: After MPTP administration, the percentage cell death in C57Bl/6J neurons is significantly reduced when cultured upon SWR/J neurons. Conversely, SWR/J neurons are significantly more vulnerable when cultured upon C57Bl/6J glia. B: After MPP^+ administration, the percentage cell death in C57Bl/6J neurons is significantly reduced when cultured upon SWR/J neurons. The amount of cell death in SWR/J neurons is 4× greater when cultured upon C57Bl/6J glia than SWR/J glia.

1996; Gerlach et al., 1991; Glover et al., 1986). Previous studies have shown that MPP^+ is the agent responsible for the cell death seen after administration of MPTP (Gerlach et al., 1991; Marsden and Jenner, 1987). Treating our cultures with 50 nM MPP^+ resulted in cell loss that was virtually identical to the MPTP-treated cultures (Table 1, Fig. 2A). Since MPTP at nonsaturating amounts is converted to MPP^+ at a 1:1 ratio (Rollema et al., 1985), we conclude that conversion of MPTP to MPP^+ via monoamine oxidases produced in glial cells is not the strain differentiating variable.

DISCUSSION

Our results clearly show that in vitr , glia are crucial in ascribing the sensitivity of neurons aft r either MPTP or MPP^+ administration. Previous studies have shown that monoamine oxidases rapidly convert the MPTP protoxin to the toxic MPP^+ (Di Monte et al., 1996; Ransom et al., 1987) at a 1:1 ratio (Rollema et al., 1985). Administration of MPTP to mice lacking MAO-B showed that SNpc neurons did not die (Shih and Chen, 1999) further demonstrating that MAO conversion of MPTP to MPP^+ is necessary to cause cell death.

If the conversion of MPTP to MPP^+ is the only role for glia in toxicity, with saturating amounts of MPTP, one would predict that a more dense glial mat would provide more MPP^+ to neurons and result in greater neuron loss. This is not the case for the chimeric cultures, in which glia proliferate for 3–4 weeks before neurons are added (compared with cultures in which the neurons and glia are plated at the same time and maintained for 7–10 days before the addition of MPTP). This suggests that in addition to toxification, the glia may also contribute a protective factor (Brenneman et al., 1997; Bronstein et al., 1995; Hou et al., 1997), which must be considered in an additive fashion.

In order to bypass the MPTP-to-MPP^+ conversion in glia, MPP^+ was directly added to the chimeric cultures. If the strain differences seen after administration of MPTP, both in vivo and in vitro, were strictly attributable to conversion of this toxin, one would have expected there to be no differences in cell death among all of the culture conditions. Our results, however, show that the levels of SN neuronal death after addition of MPP^+ are equal to that observed after MPTP administration. This result further demonstrates a role other than direct monoamine oxidase conversion from MPTP to MPP^+ for glia.

Once in the nervous system, the mechanism of action of MPP^+ is complex. MPP^+ is incorporated into neurons through the dopamine transporter leading to an inhibition of the enzymes of the mitochondrial respiratory chain (Complex I) (Han et al., 1999; Mizuno et al., 1988), depletion of ATP (Cassarino et al., 1999), disturbance of Ca^{2+} homeostasis (Frei and Richter, 1986), and formation of free radicals (Chiueh et al., 1994; Di Monte et al., 1986; Sriram et al., 1997). While each of these factors play a role in the toxicity of MPP^+, our results show that glial cells are able to modify th dopaminergic cell loss in the SN. This is illustrated by our finding that glia from a resistant mouse strain are able to reduce the percentage neuronal loss in a sensitive mouse strain by a factor of 4. Similarly, glia derived from a sensitive mouse strain increase the amount of neuronal cell death in a resistant mouse strain by a factor of 3.

Several possibilities exist as to how glial cells modulate the response of neurons to MPP^+. One might posit that the glia regulate the amount of MPP^+ that is either available t or tak n-up by the dopaminergic neurons (Kopin, 1992). However, in our in vitro system, MPP^+ is added at identical concentrations in all of the xperimental conditions, liminating variability in the amount of drug that is present within th extrac llular fluid. Once MPP^+ is present in the neuronal environ-

ment, it is internalized by the dopamine transporter (Gainetdinov et al., 1997; Pifl et al., 1993). One study examining dopamine transporter function demonstrated no differences in transporter kinetics (Womer et al., 1994) between two strains of mice that we have shown to differ in MPTP susceptibility (Hamre et al., 1999). In a similar vein, no polymorphic changes were detected in the dopamine transporter of several populations of humans with or without PD (Higuchi et al., 1995; Leighton et al., 1997; Mercier et al., 1999). These findings suggest that inherent differences in MPP^+ transport would not explain the strain differences seen after MPP^+ exposure. In addition, our chimeric experiments demonstrate that the neurons from a SWR/J mouse are not inherently resistant, since a higher percentage of them die when cultured in the presence of sensitive C57Bl/6J glia. These results strongly suggest that the glia can modify the neuronal response to MPP^+ after the initial insult.

Several recent articles have suggested that the glial cells can contribute directly to the toxic effects of MPTP, primarily through the action of free radical mediators such as inducible nitrous oxide synthase (iNOS) (Hirsch et al., 1998; McGeer and McGeer, 1998; McNaught and Jenner, 1999). In this model, administration of MPTP leads to a rapid gliosis (Schneider and Denaro, 1988), followed by an increase in the production of iNOS (Zietlow et al., 1999). iNos has been shown to interact with free radicals to produce peroxynitrite, which can interact with tyrosine residues in cellular proteins and inhibit neuronal signal transduction (Ferrante et al., 1999; Kuhn et al., 1999). Further proof for the actions of iNOS come from Liberatore and colleagues (Liberatore et al., 1999) who showed that mice lacking the gene for iNOS were significantly less susceptible to cell death compared with control littermates. In a model of iNOS action that extends the role of glia, Hirsch and Hunot (Hirsch and Hunot, 2000) suggest that MPTP acts directly to induce cytokines which in turn activates iNOS. The iNOS is then released from the glial cell to act upon dopaminergic neurons, inducing damage within these cells. Although differences in iNOS levels have not been examined in different strains of mice, it is possible that this pathway could underlie some of the differences in toxicity that occur after administration of MPTP or MPP^+.

Another possibility is that the glial cell, rather than serving as a negative factor in injury paradigms, would act as a protective factor providing a buffering capacity. In this model, the MPP^+-induced increase in free radicals could cause a multitude of responses: including the release of dopamine, reactive dopamine adducts (Ali et al., 1994; Hastings and Zigmond, 1997; Liberatore et al., 1999) and hydroxyl free radicals (·OH) (Wu et al., 1993) into the cellular environment. Extracellular neuronal dopamine has been shown to increase the stress-related protein hemeoxygenase-1 (HO-1) (Schmidt et al., 1999). HO-1 breaks down heme, which results in the release of iron and carbon monoxid (Maines, 1988). Both compounds have been shown to increase th formation of ROS through their interactions with dopamine adducts or induction of mitochondrial stress, respectively (Ibi et al., 1999; Michel et al., 1992; Zhang and Piantadosi, 1992). Once these compounds are released from the neuron, the glial cell can act to detoxify these reactive oxidative species (ROS) in the local environment (Hirsch et al., 1998; McNaught and Jenner, 1999).

Several studies have shown that astrocytes can confer neuronal protection by synthesizing and releasing the free-radical scavenger glutathione and/or its precursors glutamate, cysteine, and glycine (Dringen et al., 1999; Drukarch et al., 1998; Wang and Cynader, 2000). In addition, glia, unlike neurons, are able to use cystine as well as cysteine for the production of GSH (Sagara et al., 1993). Because GSH levels are lower in the SNpc of PD patients, this may be an important function specifically for astrocytes in the substantia nigra. The efficiency of glial cells to produce or maintain levels of glutathione in different strains of mice (Hatakeyama et al., 1996; Tanaka et al., 1991) may be an important factor in the pathogenesis of dopaminergic neuron loss in experimental models of PD.

The differences in strain response to xenobiotics may reside in a combination of positive and negative factors that can interact, resulting in an inhibition or propulsion of a feed-forward cascade leading to dopaminergic neuronal death in the SN. One of the current therapies for PD is the transplantation of dopaminergic cells, isolated either from fetal tissue (Kordower et al., 1997, 1998) or from engineered cell lines (Cunningham and McKay, 1994; Prasad et al., 1998). While the replacement of dopamine from these cells is critical for amelioration of clinical symptoms, our results suggest that the glia present in the environment may have an effect on the long-term survival of these engrafted cells. As such, we propose that in, addition to dopamine-producing cells, one should consider the controlled addition of healthy "resistant" astrocytes to the transplant. Our studies also provide a rationale for the identification of glia as a prominent target for pharmaceutical intervention in neurodegenerative disorders such as Parkinson's disease.

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